Cell Plating Protocol for USF Nanofilm Plates

Supplies needed:

- Cells of Interest
- USF Nanofilm Plate
- Fibronectin (recommended: 5ug/ml working solution in PBS)
- 70% Ethanol in UltraPure Water
- UltraPure Water (sterile)
- PBS (1X, Sterile, pH7.4)

Protocol:

Sterilization and Fiber Priming:

1. Add 0.5 mL of 70% ethanol to each well of the USF nanofilm plate to be used and incubate for 10 minutes at 25°C.
2. Aspirate ethanol carefully without touching or scraping nanofilm surface
3. Add 1 mL of UltraPure Water to each well.
   - Note: Perform wash steps quickly. Nanofibers should not be allowed to dry out; drying may cause fiber clumping and alter scaffold behavior.
4. Repeat wash two additional times (total of three washes).

Plate Coating and Cell Seeding:

5. After final water wash, add 300uL of 5ug/mL fibronectin to each well.
6. Incubate at 37°C for 2 hours.
7. Aspirate fibronectin solution.
8. Add 0.5 mL of sterile PBS to each well.
9. Repeat PBS wash two additional times (total of three washes)
   - Option: Begin preparing cell so they are ready to seed after final PBS wash
10. After the final PBS wash, seed cells onto nanofilm wells at the desired cell density & volume.
    - Note: Avoid disturbing the nanofilm surface during pipetting; dispense media against the side of the well when possible.

Additional Notes:

- Ethanol incubation serves two purposes:1) sterilization of nanofilms and 2) reduces hydrophobicity to improve coating and cell attachment
- **Keep nanofilms wet at all times.** Drying may cause fiber clumping and alter scaffold behavior.
- Coating steps can be adapted for collagen, laminin, or PDL.
- It is recommended to wait at least 48 hours after plating cells to achieve optimal imaging conditions.